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Research Report

# Behavioral changes with brief exposures to weak magnetic fields patterned to stimulate long-term potentiation

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ABSTRACT

Brief whole body exposures of rats to weak (1 μT) complex magnetic fields whose patterns induce long term potential (LTP) when applied as electric current to hippocampal slices produced powerful behavioral changes. Rats exposed for 30 min before but not 30 min after hourly training sessions for spatial memory displayed impairments comparable to those elicited by complete electrode-induced saturation of hippocampal activity. Exposure to the same LTP-patterned magnetic fields after weaning during the induction of limbic seizures produced diminished learning of conditioned contextual fear during adulthood. However exposure to magnetic fields designed to simulate a "virtual" hippocampal state during acquisition of a timed inhibitory task (DRL) facilitated performance. These results show that physiologically-patterned magnetic fields can produce dramatic changes in behavior when they are applied during states associated with marked synaptic plasticity.

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## 1. Introduction

Recent elegant experiments by Ahmed and Wieraszko (2008) showed that application of 3 s on–3 s off 15 mT magnetic fields to hippocampal slices increased the amplitude of population spikes and induced seizure-like activity. The pulsed-magnetic field (PMF)-induced amplification of population spikes involved electrical synapses and neurochemical processes in addition to those which normally mediate long term potentiation (LTP). Ahmed and Wieraszko (2008) concluded that the PMF-induced balance between different hippocampal networks might resonant differently with the applied magnetic fields. In the present series of experiments we demonstrated that whole body exposure to weak (1 μT) magnetic fields patterned after LTP-evoking stimuli for hippocampal slices (Rose et al., 1988) produced conspicuous changes in learning and memory.

One important prediction of the hypothesis that activity-dependent synaptic plasticity occurs in the hippocampus is that physiological saturation of this process should disrupt encoding of new memories (Bliss and Collingridge 1993; Chen et al., 1999; Liao et al., 1995; Lynch and Baudry 1984; McNaughton 1983; McNaughton and Morris 1987; Mellor et al., 2002; Teyler and DiScenna 1987). Repeated tetanization of a single site in the perforant pathway within the hippocampal formation can affect spatial learning; however, the direction has been variable (Castro et al., 1989; Korol et al., 1993; McNaughton et al., 1986).

During the last 15 years we have found that whole body exposures for 30 min to microTesla (μT), physiologically-patterned PMFs as "theta bursts" to induce intrinsic LTP by magnetic pulses delivered through computer software produced powerful disruptions in olfactory memory traces (Stewart, 2000), fear-conditioned analgesia (Stewart and Per-

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singer, 2000), conditioned fear to context (McKay et al., 2000) and spatial association (Delparte and Persinger, 2007).

The hippocampus contributes significantly to all of these behaviors. In the McKay et al. (2000) experiments that measured the strength of contextual fear conditioning, rats exposed to intensity-matched 7 Hz or 20 Hz sine wave magnetic fields did not differ from sham field controls. Only exposure to LTP-patterned magnetic fields resulted in marked attenuation of these behaviors. This treatment accommodated 75% of the variance in the scores for "freezing" (immobility) to contextual stimuli.

Whole body exposures of rats to appropriately-patterned 1 μT magnetic fields elicited analgesia (Fleming et al., 1994; Martin and Persinger, 2003; Martin et al., 2004a,b), comparable to 4 to 5 mg/kg of morphine. The effect was attenuated with naloxone (Martin et al., 2004b), synergized by agmatine, or enhanced by the alpha-2 agonist clonidine in a dose-dependent manner (McKay and Persinger, 2003). The analgesic effects emerged only after 15 min of exposure and were clearly evident after 30 min of exposure (Martin et al., 2004b). Similar patterns applied within the mT range produced analgesia in snails that was diminished by mu-receptor antagonists (Thomas et al., 1997). Del Seppia et al. (2007) have recently published a thorough review of that literature.

In unpublished molecular signaling experiments Buckner et al. (2008) showed that the same frequency-modulated magnetic field pattern that produced analgesia in rats (Martin et al., 2004a,b) and snails (Thomas et al., 1997) and induced "spontaneous" seizures in limbic epileptic rats (Persinger and Belanger-Chellew, 1999) affected cAMP and inhibited cell growth in various cell cultures after 3 daily exposures to 1 μT intensities. The effect was conspicuous when the daily exposures were 30 min but not 15 min and approached asymptote when daily exposures were 60 min. The temporally-reversed presentation of the same magnetic field pattern did not produce this effect. Similar anisotropic temporal effects were noted for the complex cognitive experiences reported by human subjects when these fields with similar intensities were applied transcerebrally across the temporal lobes of human volunteers (St-Pierre and Persinger, 2006).

In our research we have assumed that the effectiveness of these weak physiologically-patterned magnetic fields is increased within biological systems during heterostasis such as learning or seizure induction. We consider periods of marked synaptic activity and plasticity as manifestations of heterostasis. Wallenstein and Hasselmo (1997) have shown functional transitions between epileptiform-like activity and associative memory in the CA3 region of the hippocampus. The model of Sandblom and Galvanovski (2000) indicates that stochastic cellular systems absorb weak low frequency magnetic fields resulting in enhanced signal detection in ion channels and calcium oscillators. Voltage-gated calcium channels in cell cultures have been affected by the brief application of extremely low frequency magnetic fields (Morgan-Valle et al., 1998).

We report here that whole body exposure to a physiologically-patterned magnetic field, known to induce LTP in hippocampal slices when applied as electric currents (Rose et al., 1988), interfered with the acquisition of a water maze with the same magnitude of effectiveness as direct, current-induced depolarization (Moser et al., 1998). Simultaneous exposure to this field during post-weaning seizure induction also resulted in long-term deficits in contextual fear learning. LTP is necessary for this form of associational learning. However, rats acquiring an operant task requiring timed-inhibition of responding displayed enhanced performance when the field was applied *during* this behavior.

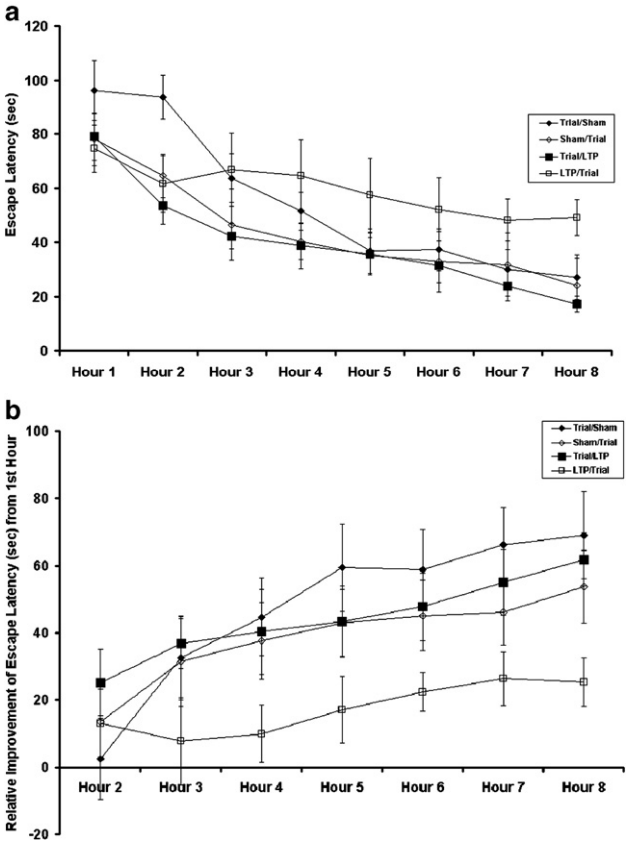


**Fig. 1 – Latencies in seconds over hourly trials for rats to escape from a water maze as a function of exposure for 30 min before or after the trials to a LTP-patterned magnetic field or to a sham condition for the same periods. (a) Raw score escape latency for the four groups ($n=36$; 9/group) for each of the 8 trials, (b) The relative time of improvement (in seconds) over the subsequent 7 h compared to the first hour (trial) for rats from the four conditions. The rats receiving the LTP-patterned magnetic field before each trial (dark square, Trial/LTP) effectively did not learn as indicated by the flat slope. Vertical bars indicate standard errors of the mean.**

## 2. Results

### 2.1. *Simulation of interference of maze learning by electrode saturation with whole body LTP-patterned magnetic fields*

We replicated the exact behavioral testing of Moser et al. (1998) except that we substituted the direct current-induced saturation by electrodes with whole body (non-invasive) exposure to

1 μT, LTP-patterned magnetic fields adapted from Rose et al. (1988). They had employed this temporal configuration as directly applied electric current (rather than magnetic fields) to produce LTP in hippocampal slices. Our hypothesis that whole body exposure to appropriate, temporally-patterned magnetic fields should simulate their effect was supported.

Fig. 1a shows the means and standard errors of the mean for the time to reach the platform (escape latency) required for the groups of rats that were exposed (within a plastic chamber in which they were free to ambulate) to either the sham or LTP-patterned magnetic fields for either 30 min before or 30 min after the 8 successive training periods (once per hour) in the water maze. The duration of the LTP pattern (Fig. 2a) was 200 ms.

Two way analysis of variance with one within subject level (8 successive hourly tests) and one between subject level (the four treatments) showed a statistically significant interaction between treatments and time ($F_{21,224}=1.81$, $P<0.05$, $eta^2=0.15$). Although the trend for the group that received the LTP-field before the hourly trials to display impairment was evident by the fourth or fifth hour, *post hoc* analyses indicated the effect was statistically significant by hour 8.

When individual differences were accommodated by dividing the latencies for all subsequent hourly performances by the first performance, the minimal learning in the group that received the LTP-patterned magnetic field before the subsequent training was clearly obvious. Fig. 1b shows the net improvement in seconds to reach the platform over the hourly testing trials for the four groups ($F_{18,192}=1.81$, $P<0.05$, $eta^2=0.13$). This behavioral pattern is almost identical to that reported with intrahippocampal saturation by electrodes by Moser et al. (1998).

### 2.2. *Long-term interference with contextual fear conditioning*

That exposure to this specific LTP-patterned magnetic field (Fig. 2a) during a period of marked physiological heterostasis, such as induction of epileptic seizures, produced permanent changes in behavior that are strongly associated with hippocampal organization can be inferred from the results shown in Fig. 2c. When tested as adults (120 days of age) the rats that had received only 1.5 h of exposure to the LTP-pattern during the development of the seizures and following treatment with acepromazine as weanlings showed a marked impairment of contextual fear conditioning compared to controls exposed to this magnetic field or to those seized, injected with ketamine, and exposed to this same field.

Whole body application of the Yun et al. (2002) magnetic field equivalent (see Fig. 2b) once every 5 s for the same duration (1.5 h) as the Rose LTP pattern (Fig. 2a) following the inductions of the seizures and treatments with acepromazine did not result in any significant impairment in adult contextual fear learning. This indicated that only a specific physiologically-patterned magnetic field affected the learning.

Fig. 2 also shows that rats in which identical seizures were induced when they were weaned but who received ketamine (100 mg/kg) instead of acepromazine and were exposed to one of the two patterned magnetic fields did not differ from controls. One-way analysis of variance revealed statistically

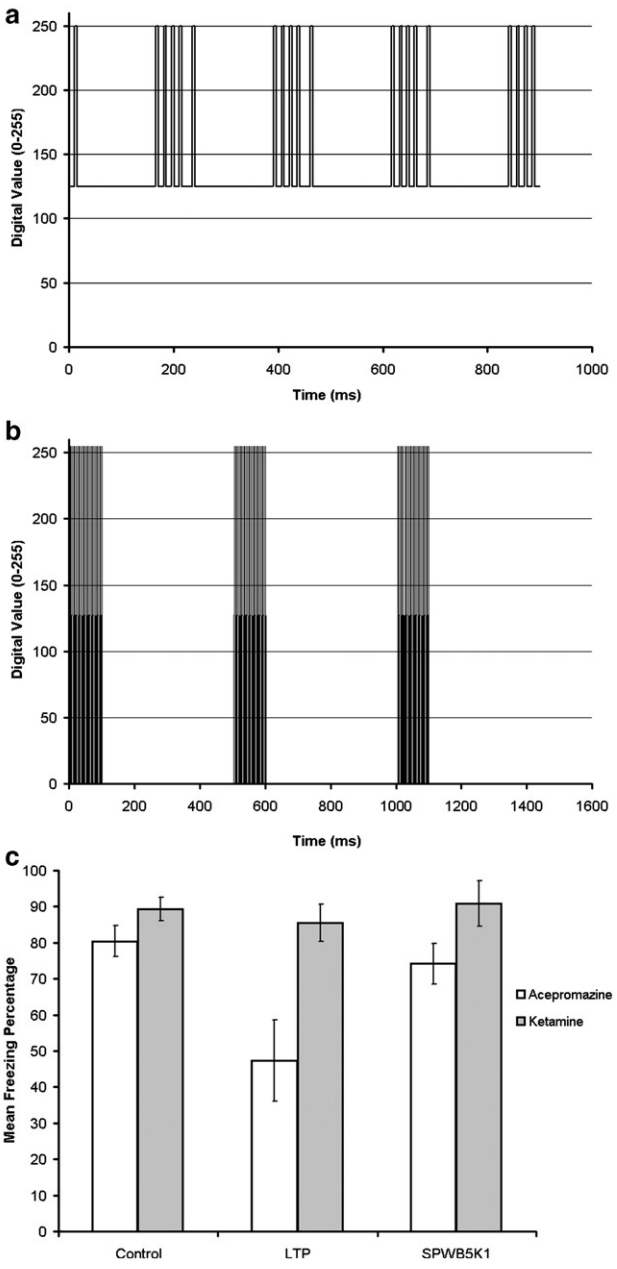


**Fig. 2 – The effects of LTP-inducing magnetic fields during induction of epileptic seizures upon consequent adult learning behavior. (a) The primary shape of the LTP-patterned from Rose et al. (1988) that generated the effects in all three experiments. The vertical axis (127 to 250) refers to the amplitude of the pulse (0 to +5 V delivered to the solenoids), (b) the complex SPWB5K1 (sharp wave-like burst stimulation, 5000 ms interstimulus interval, 1 ms point durations) pulse digitized from Yun et al., 2002); the vertical axis 0 to 250 refers to −5 V to +5 V delivered to the solenoids, and (c) mean percentage of freezing responses in the conditioned context for adult rats during the test day after one day of pairing. They had been seized at 21 days and treated with either acepromazine or ketamine while they were exposed for 1.5 h to either the LTP or SPWB patterned magnetic field (Total $n=72$; $n=12$/group). Vertical bars = SEM.**

significant group differences [$F(5,66)=5.44$, $P<0.001$; $eta^2=0.29$]. *Post hoc* analyses indicated this difference was primarily due to the behavior of the rats that received the acepromazine and Rose et al. (1988) LTP-patterned magnetic field after the seizure induction compared to all other groups that did not differ significantly from each other.

### 2.3. *LTP-patterned facilitation of ongoing timed inhibitory behavior*

The third experiment was to discern if whole body exposure to the LTP-patterned magnetic field *during* the acquisition of a timed-task would facilitate that behavior. In other words would the application of the magnetic field in real time produce a virtual neuromatrix (Fournier and Persinger, 2004) that would compensate for structural deficits due to damage or disease?

The most conspicuous behavioral effect occurred when the LTP-patterned field was presented once every 4.5 s during the 30 min of training on the first day of the shift from DRL 6-s to DRL 12-s. As shown in Fig. 3a, a normal (non-seized) adult rat displayed the usual marked increase in response-to-reward ratios typical of a schedule shift for this paradigm (Persinger et al., 1976, 1978). However an adult rat that had been seized after weaning and given acepromazine displayed quicker adaptation in the presence of the field as inferred by the lower response-to-reward ratios. These seized rats inordinately displayed increased response-to-reward ratios in DRL settings that markedly exceeded normal animals (Harrigan, Peredery and Persinger, 1990). Responses of a representative example of an adult rat (seized after weaning but given ketamine) while being exposed to the magnetic field LPT pattern during the same DRL contingencies are also shown.

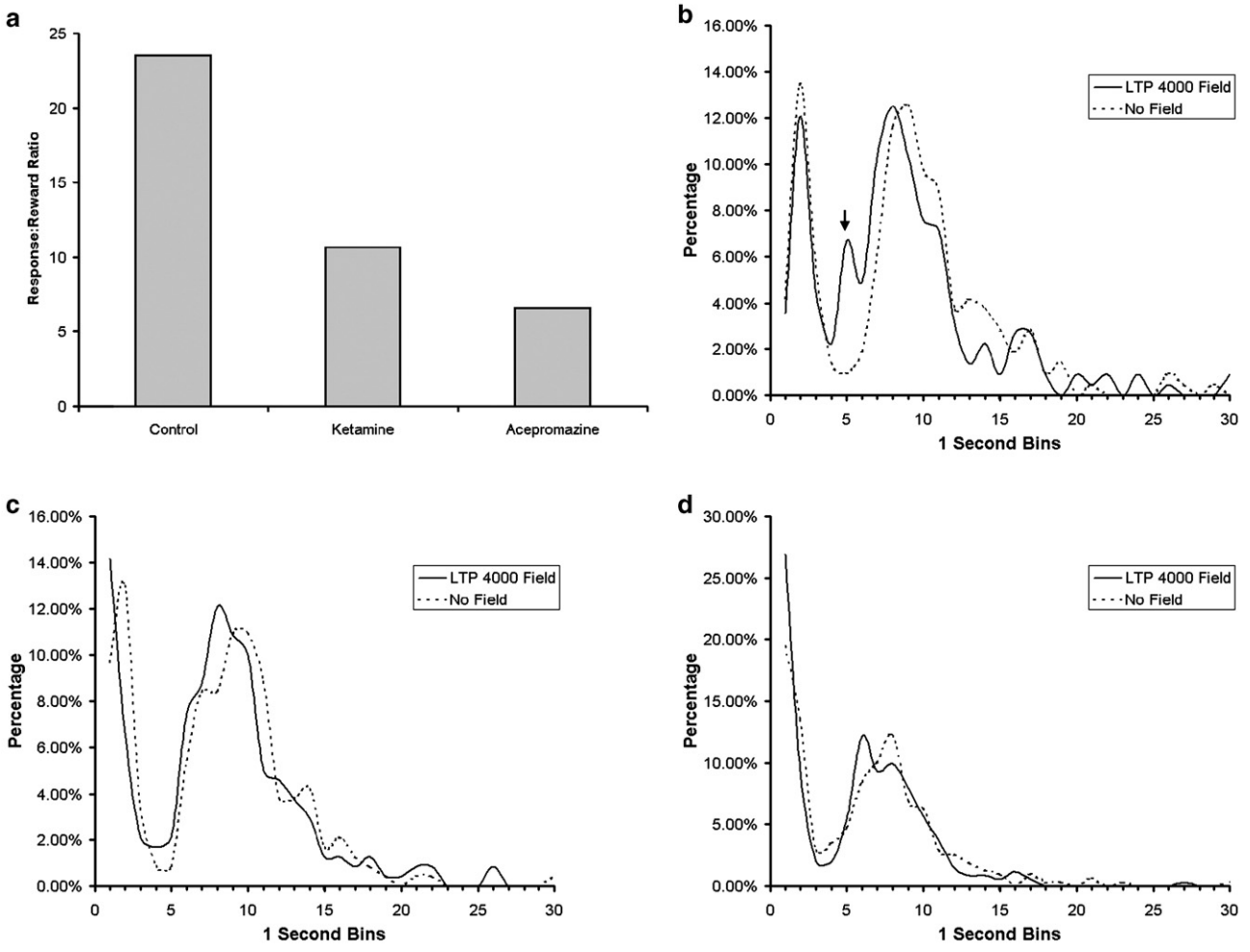


**Fig. 3 – The effects of the same LTP-patterned magnetic field (LTP-4000) while responding to demands of a DRL operant schedule. (a) Examples of ratios of numbers of responses per reward during the first session of the shift from a DRL 6-s to DRL 12-s while being exposed to the LTP-magnetic pattern for a normal rat, one which had received ketamine after seizure induction and one that had received acepromazine after seizure induction. (b) Example of percentage of inter-response times (1 s bins) for the same adult rat (seized plus acepromazine at 21 days) during the days after the DRL 6-s to DRL 12-s shift for sham field day (dotted lines) and when the LTP-magnetic field was applied while learning (solid line); note the conspicuous increase in power (arrow) within the range of the interstimulus interval of the applied LTP-magnetic field (4 to 5 s), (c) IRT distributions for a rat that had been seized and received ketamine during a no field and LTP-magnetic field day, and (d) IRT distributions for a normal rat during a no field and LTP-magnetic field day.**

The frequency distributions for the IRTs (inter-response times) as a function of 1 s bins clearly show the effect of the applied magnetic field. An increase in "power" (more proportional IRTs) is evident for an earlier seized rat (treated with acepromazine) when the LTP field was present compared to when it was not. The unique peak (indicated by the arrow) noted in an acepromazine-treated, seized rat occurred within the bin corresponding to the 4.5 s inter-stimulus-interval of the external application of the LTP magnetic field. The IRTs for a rat that was seized and given ketamine (Fig. 3c) and a normal non-seized rat (Fig. 3d) did not show the increased proportions of IRTs within the 4 to 5 s bin (the interstimulus time of the LTP field) shown by the acepromazine-treated rat (Fig. 3b).

## 3. Discussion

Three experiments demonstrated that brief whole body exposures to a specific temporal pattern of magnetic fields generated through different devices elicited robust changes in behavior. The intensities of the magnetic fields were within the range frequently encountered within both domestic and industrial settings. The strengths of these fields were also similar to those generated from a variety of electronic devices inserted over or within the ear while listening to software-generated music or general information.

The results confirmed our hypothesis that the magnitudes of the energies required to interfere with LTP-associated memory consolidation may be much less when applied as whole-body, physiologically-patterned magnetic fields than those generated by direct electrical stimulation. This hypothesis is an extension of our contention that the more physiologically-patterned the applied magnetic field the less the intensity required for that field to be effective (Martin et al., 2004a,b). In the present study the effect size, the amount of variance explained by the pre-exposure to a LTP-patterned magnetic field, was comparable to the changes reported by Moser et al. (1998) who saturated the hippocampus between trials with currents induced by electrodes.

The separation of escape latencies for high frequency and low frequency saturation by direct implanted stimulation in the Moser et al. (1998) study occurred between the fourth and fifth block of trials which would be equivalent to approximately hour three in the present study. The magnitude of the impairment in spatial learning due to whole body exposure to the LTP magnetic field before hourly testing, more clearly shown in Fig. 1b, simulated that effect.

The group that received the pre-exposures to the LTP magnetic field displayed minimal improvement over the next 7 hourly trials compared to the other three groups that did not differ significantly from each other and continued to improve over the hourly sessions. Although we had no direct measurement of saturation, the marked similarity of the effect size as well as the temporal pattern of the effects of the procedure of Moser et al. (1998) and our applied fields suggests they both may have saturated hippocampal LTP.

The order of the presentation of the LTP-patterned field was critical for the effect. Exposure for the same duration to the same LTP-patterned magnetic field after the hourly testing did not impair swimming-maze performance in subsequent sessions. The rats in the group exposed to this procedure did not differ significantly from either of the two sham-field (before or after trials) groups. Only the rats exposed to the LTP-patterned magnetic field for 30 min before the hourly training showed the significant interference with spatial learning and memory. This disruption was produced after only about 1200 pulses (3, 30 min exposures over 3 h).

This temporal asymmetry indicates that the interference with spatial learning by the applied LTP-patterned magnetic field for 30 min may have saturated the intrinsic hippocampal LTP that was associated with the representation of experience during the subsequent 10 to 15 min of testing within the maze. Thus, the LTP-patterned magnetic field appears to have prevented or attenuated the representation of spatial learning (memory) rather than interfered with its consolidation. Toni et al. (1999) found that pharmacological blocking of LTP prevented the formation of multiple synapses that was associated with accumulated calcium in dendritic spines.

The exposure to the "hippocampal" LTP magnetic field when rats were seized as weanlings produced long-term alterations in brain function. When they were tested in a conditioned fear ("anxiety") setting as adults, they showed less memory for the task than did the controls. The importance of a normally-functioning hippocampus for this specific contextual task is well known (McKay and Persinger, 2004).

That the effect was specific to a particular LTP-pattern was indicated by the normal performance displayed by the rats that were seized and also administered acepromazine but exposed instead to a different LTP-pattern designed to affect this process within the entorhinal cortices at the primary origin of the perforant pathway (Yun et al., 2002). On the other hand the LTP-fields had no effect on rats that were seized and treated with ketamine.

This absence of behavioral deficits in rats given ketamine after induction of seizures with lithium/pilocarpine is consistent with the known prophylactic effects of ketamine (Fournier and Persinger, 2004; McKay and Persinger, 2004). Post-seizure administration of ketamine does not prevent damage but alters the pattern of the neuronal dropout and increases the brain's sensitivity to otherwise ineffective magnetic fields (McKay and Persinger, 2006). The phenomenon reiterates the potential myriad of unknown synergisms between precisely-patterned electromagnetic fields and pharmaceutically-mediated changes within the brain (Thomas et al., 1979; Whissell and Persinger, 2007).

Simultaneous exposure to the LTP-patterned magnetic fields while the rats were learning to inhibit responding after a shift in the schedule increased the accuracy of performance. The improvement was inferred by the decrease in non-rewarded responses that are typically displayed by normal rats when the duration to inhibit responding was shifted from 6 s to 12 s. The IRT profile for the acepromazine rats, which usually fail to acquire this task (Harrigan et al., 1990), was consistent with a potential entrainment by the interstimulus interval of the applied LTP magnetic field. In fact conspicuous peaks in IRTs occurred within the bins corresponding to the inter-stimulus-interval of the applied pattern. We have seen at least one example of such "entrainment" by applied burst-firing magnetic fields upon Stage 2 ("spindles") in a patient

with markedly elevated partial complex epileptic symptoms subsequent to a mild closed head injury (Persinger et al., 2009).

Stimulation of the hippocampus during the peak of the theta rhythm yields LTP but long-term depression when delivered during the trough (Hyman et al., 2003). Electrical stimuli patterned after theta-rhythms, such as the one employed in our experiments, can produce multiple forms of LTP which is dependent upon calcium influx through L-type voltage channels (Morgan and Teyler, 2001). Morgan-Valle et al. (1998) demonstrated the effects of 60 Hz magnetic fields upon L-type $Ca^{+2}$ channels in chromaffin cells.

## 4. Conclusion

Our results offer an alternative method for studying LTP and other electrophysiological and electromagnetic correlates of learning and memory that does not require surgery or the invasiveness of implantations. This alternative not only facilitates a more humane inclusion of animals in this type of research but allows more frequent employment of the same rats for different experiments.

Modern computer technology has contributed to the rational and thoughtful creations of physiologically-patterned magnetic fields whose complexities may be unlimited. This is important in light of the research of Yun et al. (2002) who found that different layers of the entorhinal cortices and subsections of the hippocampal formation may display different but specific optimal parameters by which LTP within these regions can be evoked.

If this concept is generalizable, then the previous impedances of "focusing" applied weak magnetic fields to specific three-dimensional points in brain space would be moot. By applying throughout brain space complex magnetic fields whose temporal patterns most approximate those generated within a specific brain structure temporal resonance and "stimulation" within this area might be achieved (Ahmed and Wieraszko, 2008).

This procedure would be analogous to the release of a hypophyseal stimulating hormone. Although it would be distributed throughout the body and be proximal to every cell the hormone would only affect those cells that expressed specific receptors "structurally" resonant with its specific molecular pattern. Whereas chemical effects are mediated by their specific spatial (molecular) patterns applied electromagnetic field effects might be mediated by their specific temporal patterns (Martin et al., 2004b).

## 5. Experimental procedures

In the three experiments the same LTP magnetic field with the same point durations and temporal patterns generated from the computer software were applied to three different arrangements of solenoids to produce the magnetic fields. Direct measurements of the magnetic patterns with a small solenoid (reed switch) placed within the exposure cages demonstrated the strong fidelity of the signal generated between the solenoids. This was completed by directing the induced current to an electroencephalographic strip chart recording as well as by listening to the "sound equivalent" of the magnetic field generated by the solenoids by connecting a telephone microphone jack (Radio Shack) to an acoustic amplifier. The patterns were effectively identical to that shown in Fig. 2. The generation of the signal through the digital-to-analogue converter into the paired solenoids did not produce any audible artifacts.

The computer program transformed a column of numbers in the file between 0 and 255 (0 to 126=negative polarity, 127=0 polarity, and 128 through 255=positive polarity that produced the pattern over time. The time (point duration) for each successive "number" and hence each voltage was activated was 1 ms. The outputs from the computers were connected to custom-constructed digital-to-analogue converters from which the current was delivered to solenoids. Different computers, d-to-a converters, and solenoid configurations were employed with each of the three experiments. Only the LTP-patterned magnetic field remained the same. There was never any evidence of heating of these small coils or subtle sounds even after 1 h continuous delivery of the ±5 V from the digital-to-analogue converters and through the insulated wires that wrapped the iron cores.

Experiment 1 was designed to discern if whole body exposure for 30 min to a magnetic field patterned to induce LTP either before or after successive hourly trials in the Morris Water Maze could impair spatial learning to the level evoked by direct intracerebral electrode stimulation as described by Moser et al. (1998). They suggested that saturation would be most robustly achieved by an electrode array that straddled afferent pathways and by a stimulation protocol that consisted of multiple tetanization episodes with cathodal stimulation at different cross-sectional sites.

Their procedures provided a more pervasive saturation of hippocampal LTP and evoked a powerful disruption in spatial learning. We had reasoned that if spatial pervasiveness and the optimal *physiologically* patterned stimulation were essential for this saturation, comparable disruptions in spatial learning could be induced by whole body (brain) exposure to weak magnetic fields within the microTesla range of intensity if the temporal structure simulated the conditions known to induce LTP.

The LTP pattern generated between the two solenoids consisted of a primer 5 ms pulse followed 151 s by 3, 5 ms pulses each separated by 11 ms. This pattern (1+3) and an interval of 130 to 170 ms between the primer and pulse train was considered optimal to generate reliable LTP in hippocampal slices when applied as electric current (Rose et al., 1988; Chen et al., 1999; McKay and Persinger 2000; Stewart and Persinger 2000).

A total of 36 male albino Wistar rats between 3 months and 7 months of age were tested in 9 blocks (4/block). A block consisted of 1 rat in each of four groups: testing/sham; sham/testing; testing/LTP, and LTP/testing. The basic procedure was the following. Each rat was exposed to either the sham field or the 1 microTesla LTP-patterned field either for 30 min before or for 30 min after testing in the Morris Water Maze. This occurred 8 times over 8 successive hours. Each hourly testing consisted of 3 trials with a maximum of 2 min allowed for each trial.

The rats were exposed to the sham or LTP-patterned field in a separate room (Martin et al., 2004b) in a plastic cage that

allowed ambulation. An IBM XT computer employing Complex2 software (Martin et al., 2004b) was employed to generate the fields by converting a series of numbers between 127 (zero polarity) and 255 (+5 V). Time between each pattern was 4.5 s. This resulted in a total of 400 "stimulations" per exposure. Rats exposed to the sham fields were placed in the same container with all of the equipment operating except that the field was not activated.

The 25 cm×25 cm×25 cm chamber in which the rats received either sham field treatment or the LTP-magnetic field either before or after maze training was identical to that reported by Martin et al. (2004a,b). Two large nails (25 cm long, 1 cm diameter, about 140 g) each wrapped with 1050 turns of 20-gauge insulated wire, were opposed to the opposite sides of the plastic exposure container along the horizontal plane. The linear axis of the nails was approximately 4.5 cm above the surface of the plastic floor that was covered by one-quarter inch corncob bedding. The peak strength of the field 2 cm from either pole within the bisector between the poles was 2.5 μT. At radii of 4, 6, 8 and 20 cm from the pole, the strengths were 1.7, 1.0, 0.5 and 0.3 μT, respectively.

The field strengths were measured with a Metex N380 meter coupled to a magnetic sensor (140-3-60-1499, Electric Field Measurements, Rt. 183, W. Stockbridge, MA, 01266). About 60% of the cross-sectional area occupied by the body of the rat displayed an intensity of about 1 μT. We have found the spatially heterogeneous magnetic fields are more behavioral effective than homogeneous magnetic fields of comparable intensities produced by Helmholtz or Merritt coils (Martin and Persinger, 2003).

In experiment 2, a total of 72 male litters mates from 17 different litters served as controls or were injected subcutaneously between 21 and 24 days of age with 3 mEq/kg of LiCl and then 4 h later with 30 mg/kg of pilocarpine. The rats were then placed immediately into a magnetic field configuration (same size but different plastic chamber) that was different than the one employed in Experiment 1. In this instance the magnetic fields were applied in three-dimensional Cartesian space. The LTP-pattern was identical to the one applied in Experiment 1.

Details on the physical dimensions and application of the electromagnetic fields can be found elsewhere (McKay et al., 2000). Effectively the magnetic field was applied through small solenoids (reed switches) successively for 0.5 s through the X, Y, and Z plane before it was applied simultaneously in all three planes. The input from the digital-to-analogue converter was programmed to shift the input to successive pairs of solenoids.

Immediately after the onset of Racine Level 5 seizures (rearing, rapid forepaw clonus, falling), which occurred between 5 and 8 min after injection of the pilocarpine) the rats (still in either the sham or magnetic field condition) were injected subcutaneous with either 25 mg/kg of acepromazine or 100 mg/kg of ketamine. The injections were required to facilitate survival (Persinger et al., 1993). Total field exposure was 1.5 h. Rat pups were immobile within 2 min after the injection of either ketamine or acepromazine. The intensity of the field within 60% of the exposure area was 1 μT.

During the first blocks in this experiment we were concerned that the obvious impairment in adult contextual fear conditioning from earlier periseizure-exposure to the LTP-magnetic field might be non-specific to its temporal pattern and due to simply the presence of any magnetic field. Consequently in subsequent blocks litter mates of rats were exposed to no (sham) field, the same LTP we had used previously, or to a qualitatively different temporal pattern of Sharp Wave-like Burst Stimulation (SPWBS) designed after Yun et al. (2002). They had induced large degrees of LTP by directly applying this pulse within Layers II, III of the entorhinal cortices.

We converted the temporal patterns of their electrical pulses by our programmable software to the equivalent magnetic field whose fidelity was verified by directly measuring the magnetic field pattern within the exposure chamber. This is a different pattern that would be expected to have a lesser effect on hippocampal function if the magnetic pattern simulated their procedures. In total there were 6 groups with 12 rats per group that were tested over an 8 month period.

After the exposure the rats were placed in maintenance cages (3 per cage) until they were ready for testing around 120 days of age. At that time rats were tested singly. The rats were placed in a 28 cm×20 cm×20 cm modified operant chamber, composed of aluminum sides with a plexiglass front and back and a hinged plexiglass top. The floor of the chamber was formed by 18 steel rods, 1.5 cm apart. The subjects were transported from their home cages in large plexiglass cages and placed within the fear conditioning chamber.

Rats were given 3 min to habituate and pre-shock motor activity was recorded. Motor activity was scored as a midline crossing, with forward movement of the whole body across the midline (same procedure as McKay and Persinger, 2003). Three foot shocks, 2 s in duration with an intensity of 0.5 mA were given at 60 s intervals. The rat remained in the chamber for 60 s before it was returned to the home cage.

After the rat was returned to the home cage, the fear conditioning chamber was wiped with a diluted vinegar solution before the next trial following the accepted procedures for open field testing (Persinger, 1969). Twenty-four hours after conditioning, the subject was placed back into the chamber for 8 min. Defensive freezing and grooming during the contextual retention test was measured with a time sampling of one observation every 8 s for a total of 60 observations. Percent time freezing during retention was calculated.

In experiment 3, normal rats and rats with histories of epileptic seizures (and brain damage) but treated immediately after seizure-induction with either acepromazine or ketamine were exposed to the LTP-patterned magnetic field while they were training for 30 min per day on either DRL (differential reinforcement of low rates of responding) 6-s or DRL 12-s schedules. This meant that the rat was required to inhibit a bar press for 6 s or 12 s, respectively, to receive a reward; a response before that time would result in a delay for another 6 s or 12 s.

A total of 8 male Wistar rats with histories of being normal or seized just after weaning (as in experiment 2) and being treated with either the same dosages of acepromazine or ketamine were selected as subjects in a differential reinforcement of low rates of responding (DRL) behavior paradigm. The subjects were food deprived to approximately 85% of free

feeding weight and placed within the DRL chamber. In order to train the subjects to press a lever a continuous reinforcement (CRF) schedule was used for two days prior to testing.

The criteria for task acquisition was 50 bar presses per day for two consecutive days. After the CRF training, the rats were given a DRL latency of 6 s. The DRL 6-s was carried out over 10 successive days immediately following the CRF training. After that time an LTP field was applied to the subject while in the DRL chamber through a different pair of solenoids (Fleming et al., 1994) that were constructed from large nails wrapped with wire similar to Martin et al. (2004b). This was a different exposure apparatus than the ones used in the first two experiments.

The field strength within the operant chamber occupied by the rat was between 0.5 and 2 μT (average 1 μT). The procedure was repeated for 18 days, with the LTP field applied the first day, followed by no field. After the 18 days, the schedule was changed from DRL 6-s to DRL 12-s and the LTP-patterned fields were given over a period of 6 days. On the first day of field exposure, the LTP field was configured so a 4.5 s time interval separated each presentation of the LTP pattern. During the following day no field was applied.

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